

**HOST SWITCHING IN *CULEX* MOSQUITOES:  
EFFECTS OF PHOTOPERIOD AND AGING  
ON HOST CHOICE**

by

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## ABSTRACT

*Culex pipiens* has long been recognized as an important enzootic vector of arboviral diseases throughout the mid-Atlantic U.S. Recently, the role of mosquitoes in the *Cx. pipiens* complex as bridge vectors has been debated. West Nile Virus has spread throughout the continental U.S. and the threat of arboviral introduction is a constant reality. Therefore, the determination of the vectorial capacity of *Cx. pipiens* as a bridge vector is vital to understanding local disease transmission cycles now and in the future. This study aimed to investigate the effects of aging and larval photoperiod exposure on the feeding behavior of *Culex pipiens*. This was explored in the lab using a host choice chamber with Japanese quail and golden Syrian hamsters as possible host choices. Mosquito cohorts were reared under specified photoperiod patterns to mimic naturally occurring seasonal changes in daylength: 15L:9D; 14L:10D; and 13L:11D. Larval mosquitoes reared at 15 hours of daylight, which was set to mimic the local peak summer daylength, showed a statistically significant preference for Japanese quail. This preference was not seen when evaluating mosquitoes reared at 14 or 13 hours daylight, which supports the hypothesis that early season mosquitoes show a preference for avian hosts which is lost later in the season. Adult mosquitoes were tested in the host choice chamber at different ages (measured in days post emergence). The tendency of mosquitoes to enter any side host chamber

increased with greater number of days post emergence. There was no correlation with host choice over aging. Host-baited trapping was performed in the field using Japanese quail and golden Syrian hamsters resulting in a statistically significant preference for hamsters early in the season, which does not support the hypothesis that early season mosquitoes prefer avian hosts. It is important to note that large discrepancies in the probability of host choice were apparent among field sites and between seasonal years. A higher percentage of mammalian bloodmeals (69%) was found in late summer using ELISA analyses of bloodfed *Cx. pipiens*, which again supports the hypothesis that a preference for avian hosts is lost later in the season. The final objective was to evaluate the previously reported trend of *Cx. pipiens* feeding preference with relation to avian host abundance. Regression probabilities of ELISA showed elevated mammalian host positives coincided with periods of low bird species abundance, which corresponds with previously reported trends. Overall, these results support the overall influence of avian host availability on fluctuations in *Cx. pipiens* seasonal feeding trends. However, trends that correlate feeding patterns with aging and larval daylength exposure reported here have not been previously considered and therefore require further investigation to determine influence on *Cx. pipiens* populations in the field.



## INTRODUCTION

The introduction of West Nile virus (WNV) to the U.S. in 1999 brought resurgence in arboviral research and a reminder of the impact that invasive arboviral diseases may have on humans and domestic animals in the U.S. Arboviruses (arthropod-borne viruses) can have intricate ecological transmission cycles involving one or more arthropod vectors and several possible non-human, vertebrate hosts. Female mosquitoes require a bloodmeal for egg production and may acquire viruses from infected vertebrate hosts. After an extrinsic incubation period within the insect vector, infective mosquitoes seeking another bloodmeal can transmit viruses to other vertebrate hosts (Nasci and Moore 1998). As is the case with many encephalitic viruses endemic to North America, an amplifying animal host maintains the virus within an ecosystem. An increase in the number of infected vectors generally leads to a subsequent increase in the number of infected amplifying hosts which can result in a spillover effect, i.e. the incidental infection of hosts such as horses and humans (Nasci and Moore 1998).

Until the introduction of WNV to the U.S., LaCrosse encephalitis and St. Louis encephalitis (SLE) were the most frequently reported mosquito-borne viral infections in humans in the U.S. From 1964-2001, over 4,000 human cases of SLE were reported; however, the introduction of WNV to New York, New York in 1999 resulted in approximately 4,000 reported human infections by 2002 (CDC 2009; Herrington

2003). A peak of approximately 10,000 cases was reported in 2003 and WNV now encompasses a large geographic area including the entire continental U.S. (CDC 2009). Disease outbreaks are contingent upon several ecological and environmental factors because transmission depends on factors such as vector feeding behavior and host competence (Eldridge 1987).

Both SLE and WNV are vectored by mosquitoes of the genus *Culex*. *Culex* mosquitoes are largely associated with viral and filarial disease transmission around the world. In the mid-Atlantic region of the U.S., *Culex pipiens* has been implicated as an important vector because it can reach large numbers locally and members of this complex are associated with several diseases including WNV, SLE and eastern equine encephalitis (Fonseca et al 2004). Earlier studies focused largely on *Culex pipiens pipiens* (L.) as an enzootic vector based on data obtained from feeding preference studies (Molaei et al. 2006; Turell et al. 2005). Previous studies largely reported that *Cx. pipiens* predominantly feed on avian hosts (Crans 1964; Means 1968; Magnarelli et al. 1977; Rockett and Somers 1983; Irby and Apperson 1988; Apperson et al. 2002). Little attention was paid to studies that reported *Cx. pipiens* feeding on several mammalian hosts including humans (Murphey et al. 1967; Edman and Downe 1964; Ekis and Hagmann 1968; Savage et al. 2007).

Species in the *Cx. pipiens* complex are competent laboratory WNV vectors and occur naturally in large numbers coinciding with the timing of outbreak occurrences

(Turell et al. 2005). Infected mosquitoes are commonly collected in surveillance studies, and bloodmeal analysis has revealed feeding on both avian and mammalian hosts (Kilpatrick et al. 2006; Apperson et al. 2004). Hamer et al. (2008) reported the first collection of a WNV-infected *Cx. pipiens* mosquito that had previously fed on a human. An increased understanding of *Cx. pipiens* host feeding patterns is required to determine its full potential as an epidemic (bridge) vector of arboviruses.

### **Culex pipiens biology**

Bionomics of mosquito species often dictates their role as disease vectors. For instance, specific mosquito species reside in different habitats and exhibit different patterns of seasonal emergence and peak abundance (Clements 1992). Several characteristics of *Cx. pipiens* life history have implicated the species as an enzootic and epidemic disease vector. This species commonly breeds in anthropogenic water sources and is abundant in densely populated areas. *Culex pipiens* larvae are found in foul water sources such as rain barrels, catch basins, and ditches. The species is a nuisance biter that is highly active at dusk and dawn (Ekis 1971). *Culex pipiens* is a non-native, invasive species introduced to the U.S. centuries ago and the species is considered peridomestic because of its close association with humans (Carpenter and LaCasse 1955). Lastly, *Cx. pipiens* populations peak in mid-August, which coincides with the emergence of arboviral diseases such as WNV (Andreadis et al. 2001 and 2004).

*Culex pipiens* complex consists of: *Cx. pipiens* f. *pipiens* L., *Cx. pipiens* f. *molestus* Forskal, *Cx. pipiens pallens* Coquillett, *Cx. quinquefasciatus* Say, *Cx. australicus* Dobrotworsky and Drummond, and *Cx. globocoxitus* Dobrotworsky (Smith and Fonseca 2004). *Culex pipiens* f. *pipiens*, *Cx. pipiens* f. *molestus*, and *Cx. quinquefasciatus* are the important vector species in the mid-Atlantic region. *Culex pipiens* f. *pipiens* is commonly referred to as the northern house mosquito because its geographic range extends throughout the northern states. Similarly, *Cx. quinquefasciatus* is also known as the southern house mosquito because this species mainly occupies the southern states (Cornel et al. 2003). Hybrids of these two forms have been documented in temperate regions (Barr 1957). Interestingly, in warmer climates hybrid populations are more abundant early in the season. Alternately, in cooler climates, hybrid populations peak late in the season (Murphey et al. 1967). This pattern has important implications for disease transmission as feeding behavior has been linked to genetic variability (Fonseca et al. 2004).

The third prevalent form throughout the mid-Atlantic region is *Cx. pipiens* f. *molestus*. Morphologically, *Cx. pipiens* f. *pipiens* and *Cx. pipiens* f. *molestus* are identical, but they are distinguishable by several behavioral and physiological characteristics. *Culex pipiens* f. *pipiens* develops and mates in aboveground, spacious environments (eurygamous), diapauses over winter, and requires a bloodmeal for all egg mass production (anautogenous) (Spielman and Wong 1973). Alternately, *Cx.*

*pipiens f. molestus* is stenogamous (breeding in confined spaces), lacks the winter diapause capability, and develops an initial egg mass without taking blood (autogenous) (Spielman and Wong 1973). Because this study focuses primarily on the feeding behavior of *Cx. pipiens f. pipiens*, this form will be referred to as *Cx. pipiens* with other forms specified as needed.

### **Disease vectors**

*Culex pipiens* mosquitoes are important vectors for WNV, SLE, EEE and dog heartworm (*Dirofilaria immitis*) throughout North America (Nasci and Miller 1996). This species also vectors Rift Valley fever in Africa (Hoogstraal et al. 1979), which has been listed on the Homeland Security Bio-Defense list (DHS 2007), and *Wuchereria bancrofti* (filariasis) in Asia and elsewhere (Gad et al. 1988). The introduction of WNV to the U.S. has provided a unique opportunity to document the spread of an introduced arbovirus.

WNV has primarily avian reservoirs; the virus is spread via blood-sucking arthropods, with *Cx. pipiens* being recognized as the most important enzootic vector. Also, the virus is maintained and often amplified in the ecosystem via infected avian populations. WNV has been found in 326 different avian species, including those that are native, introduced and captive exotic species (CDC 2009). In cold and temperate climates, the risk of WNV infection ends with the first frost, as mosquitoes are no

longer abundant and able to transmit the virus. Significantly, WNV recurs throughout the northeastern and mid-Atlantic states of the U.S. each year.

There are several possible mechanisms in the transmission dynamics of WNV that may allow for annual recurrence of this virus. First, the virus may overwinter in adult, diapausing mosquitoes, which would then initiate the spread of the virus the following spring (Andreadis et al. 2001). This possibility has been supported by the finding of WNV in hibernating *Cx. pipiens* and subsequent transmission to birds early in the season (Nasci et al. 2001, Dohm et al. 2002). A second and less supported hypothesis cites the possibility of migratory birds reintroducing the virus each spring, because it has been documented that the virus is present in the ecosystem year-round in warmer climates (Apperson et al. 2002). Reintroduction of WNV into ecosystems in the northern U.S. may be a result of infected birds migrating north or recrudescence of the virus in overwintering birds. Studies have shown that permanent resident birds have higher seropositivity than migrant birds with overwintering migrants having the highest rate of seropositivity (Shelite et al. 2008). Lastly, researchers speculate at the ability of WNV to spread vertically through a mosquito population, with the eggs of infected females becoming infection sources as well (Baqar et al. 1993). It is possible that a combination of mechanisms occurs, where infective migratory birds prompt a general area reintroduction and mosquitoes spread the disease locally (Rappole et al. 2000). Finally, it has been speculated that infected birds that overwinter locally within

an ecosystem may experience a relapse of virus in the subsequent spring allowing for infected birds to serve as infective reservoir hosts (Reisen et al. 2006).

Extensive evidence supports *Cx. pipiens* as an enzootic vector for WNV. Since the introduction of WNV to the U.S., surveillance programs have repeatedly reported the collection of infected *Cx. pipiens* (Andreadis et al. 2001 and 2004; Anderson et al. 2004). Because of inconsistencies in feeding studies based on bloodmeal analysis, the state of *Cx. pipiens* as an epidemic vector is uncertain (Kilpatrick et al. 2007; Hamer et al. 2008).

The number of emergent infectious diseases has increased due to growth in international travel and commerce and changes in human demographics. This increase has been attributed to global commerce, war, political unrest, and misuse of medical technology, such as abuse of prescriptive drug treatments and blood transfusions (Morse 1995). Similarly, international trade and travel have been implicated in the introduction of invasive vector species (Lounibos 2002). It is vital to determine the ecological parameters affecting arboviral transmission, specifically vectorial capacity, as these factors may have implications for future disease introductions.

### **Feeding behavior**

Studies of insect physiology suggest that feeding behavior is dependent upon multiple signaling cascades triggered by both hormonal and sensory input (Klowden 1983). It is commonly accepted that blood seeking organisms depend on sensory

input of kairomones emanating from potential hosts, a classically - accepted driving force of host-seeking behavior. Yet feeding behavior may also be affected by extrinsic cues that are independent of the host and act on hormonal cascades (Clements 1992).

Growing concern over WNV outbreaks led to an increased interest in determining vectorial capacity of mosquito species, which largely depends on mosquito feeding behavior. Several early studies characterized *Culex pipiens* as strong ornithophilic feeders (Crans 1964; Means 1968; Magnarelli 1977; Irby and Anderson 1988; Apperson et al. 2002). Yet other studies have shown that this species feeds on mammals, including humans (Kilpatrick et al. 2006). These findings have led to a search for a more accurate determination of the vectorial capacity of *Cx. pipiens* mosquitoes in transmitting WNV to humans.

### **Seasonal Feeding Trends**

Much of the controversy surrounding the host switching behavior of *Cx. pipiens* and *Cx. restuans* pertains to the seasonal trends of host switching behavior. When switching from avian to mammalian hosts is discussed, authors often mention that mammalian feeding occurs in higher percentages later in the season (Kilpatrick et al. 2006, Andreadis et al. 2001). Kilpatrick et al. (2006) offered an interesting



hypothesis regarding the host switching of *Cx. pipiens* in Washington D.C. The authors ultimately speculated that *Cx. pipiens* feeding behavior is linked to the density of a particular avian host, the American robin (*Turdus migratorius* L.). This speculation was based on their findings that as robin densities decreased the percentage of mammalian blood meals among *Cx. pipiens* populations increased. Although it seems a viable hypothesis, studies assessing avian hosts of *Culex* mosquitoes have not consistently found American robins to be predominantly favored by *Cx. pipiens*. In fact, a study performed by Patrican et al. (2007) reported *Cx. pipiens* preferred northern cardinals (*Cardinalis cardinalis* L.) over American robins in New York. Marshall et al. (2006) linked WNV- infected *Cx. pipiens* feeding with seasonal American robin population abundance in Ohio. Data collected by Apperson et al. (2002) showed *Cx. pipiens* fed equally on northern cardinals and American robins. Savage et al. (2007) also reported on *Cx. pipiens* feeding and WNV host capacity in Tennessee. This study supported previous findings that *Culex* mosquitoes prefer American robins as blood meal sources. Savage et al. (2007) also reported larger proportions of robin blood meals in spring and early summer, consistent with previous observations of a temporal change in feeding behavior. Yet, as the authors note, American robins are present throughout the year at the Tennessee study sites (Savage et al. 2007). Therefore, it seems that the hypothesis presented by Kilpatrick et al. (2006) may be habitat-dependent.

Seasonal trends in feeding behavior, particularly in temperate areas of the mid-Atlantic have also been linked to the hybridization of *Cx. pipiens* and *Cx. pipiens* f. *molestus*. Feeding behavior is a vital difference between these two forms. *Culex pipiens* f. *molestus* is classically considered a mammalian feeder, while *Cx. pipiens* f. *pipiens* has been largely considered an avian feeder. Also, in temperate regions, hybrid populations peak late in the season which coincides with the seasonal transmission cycle of WNV. Because the two forms are morphologically indistinguishable, researchers have used microsatellite analysis to determine the genetic profile of mosquito populations (Fonseca et al. 2004; Huang et al. 2008; O'Connor 2008). While these studies have reported that hybrid forms of *Culex* mosquitoes can be present in large proportions, little evidence strongly links hybridization to seasonal trends in feeding (O'Connor 2008).

### **Biological Features Influencing Feeding Behavior**

A lack of consensus surrounding previous hypotheses on the seasonal shifts in *Culex pipiens* feeding preference has promoted increased interest in biological cues. These cues should also be correlated with the progression of the disease transmission cycle throughout the season in order to implicate *Cx. pipiens* as bridge vectors. In order for *Cx. pipiens* to prove itself a likely bridge vector, dependence on avian hosts should decrease through the season, leading to a more opportunistic feeding behavior beginning in late August. Feeding behavior may largely be affected by mating and

oviposition which involve hormonal fluctuations. Changes in reproductive state can also trigger hormonal feedback loops that alter insect behavior (Klowden 1983). Older vectors would have more time to acquire and transmit pathogens throughout a habitat. Also, most arboviral diseases, including WNV, require an external incubation period within the mosquito vector. In the case of WNV, with *Culex pipiens* acting as the vector, up to 16 days is needed for the vector to successfully transmit WNV to a new host, but this can fluctuate with respect to ambient temperature (Anderson et al. 2008). Coupled with changes in behavior associated with senescence, many authors have noted the importance of determining host choice as a function of mosquito age (Klowden and Arden 1980; Gillies and Wilkes 1965). With each gonotrophic cycle, the process of oogenesis causes irreversible changes in the genital tract of female mosquitoes (Klowden and Arden 1980). Studies often note that physiologically old mosquitoes differ in behavioral aspects. For instance, young mosquitoes may feed early in the night while parous females more often feed in the morning (Aslam et al. 1977).

Another cue that may offer a more stable pattern for seasonal shifts in feeding is related to the effects of photoperiod during larval development. Spielman and Wong (1973) speculated that mosquitoes enter a reproductively dormant diapausing state when exposed to shorter day-lengths of about 13 hours. The reproductive state of the adult was dependent on the daylight exposure during larval development.

Changes in photoperiod cycles offer more predictable patterns to alter actions such as reproduction and development unlike host availability, which can vary largely by habitat (Blum 1985).

### **Scope of Research**

This study investigated the effects of aging and larval photoperiod exposure on the feeding behavior of *Culex pipiens*. The objectives of this study were to: 1) determine the effects of photoperiod exposure during the larval growth period on host choice behavior of *Cx. pipiens*; 2) determine if a relationship between age and host feeding choice of adult *Cx. pipiens* mosquitoes can be documented under controlled laboratory conditions; 3) use a modified host-baited trapping system to ascertain seasonal host choice behavior in the field; 4) use ELISA bloodmeal analysis methods to fully determine seasonal patterns in host choice behavior of field caught *Cx. pipiens*; and 5) regularly measure avian populations at field collection sites and determine if their abundance is correlated with analyzed bloodmeals of trapped mosquitoes from these sites.

## METHODS

### Site Descriptions

Adult mosquitoes and egg rafts were collected from several locations in northern Delaware in order to maintain adequate test values and assess differences among geographically distinct areas. Host-baited traps were also set at each of these locations on a weekly basis. Before the morning collection of these traps, data on the avian population was collected by performing bird counts. All collections occurred from May to September during two successive years (2008-2009).

The Cherry Island landfill site (39°31'37.93"N; 75°31'37.93"W) in Wilmington, DE was located between the Wilmington sewage treatment plant to the north and Christina River to the south, and was used in the 2008 season. Trees and other vegetation lined the riverbank along the south end of the river, which offered a suitable place for mosquito trap placement.

The Port of Wilmington site (39°42'12.92"N; 75°31'44.10"W) replaced the Cherry Island landfill site for the 2009 season. Traps set out at Cherry Island were subjected to several incidents of human tampering and the U.S. Army Corps of Engineers shifted dredging activity, which created *Culex* breeding environments, accessible to the Port of Wilmington. This site was largely overgrown with tall vegetation and was not regularly disturbed by human traffic. The Delaware Solid

Waste Authority bordered the south end of the site and the rest of the site was bordered by marshy land along the Delaware River.

The Cathedral Cemetery site (39°44'53.24"N; 75°34'57.44 W) was located near residential neighborhoods in Colonial Heights in Wilmington, DE. This urban environment, littered with anthropogenic water sources and debris, was used in both 2008 and 2009. The area in which traps were placed abuts the CSX Railroad freight-line tracks parallel to a large storm-water drainage pipe which separated the trap location from a 65-acre cemetery.

The site in New Castle City, DE (39°40'14.37"N; 75°33'31.81"W) was located near the intersection of Wilmington Road and Glebe Lane; this site was used for both the 2008 and 2009 seasons. This small wooded area was located behind an open storage building accompanied by abandoned equipment and organic debris. In 2008, the immediate area near the trapping site was subjected to regular traffic as landscaping crews transported mulch and yard waste to and from the location. This activity did not regularly occur throughout the 2009 season.

Glenville Park, in Glenville, DE (39°42'28.49"N; 75°38'26.27"W) was the site of an industrial park used in 2008 and 2009. The site was surrounded by large plots of land that were residential areas in the past, but regular flooding led to seizure by eminent domain. Houses have been demolished from all abandoned lots and vegetative succession has occurred, but mostly tall grasses and invasive shrubs occur

there. Vegetative succession was more pronounced in the 2009 season as demolition and evacuation had largely ceased.

Lastly, a site along the woodlot on the south campus of the University of Delaware in Newark, DE (39°39'49.94"N; 75°44'43.08"W) was used in 2008 and 2009. The site was bordered by the University of Delaware woodlot to the north and west, agricultural fields to the east, and Delaware Highway 4 to the south.

Cherry Island, Cathedral Cemetery and New Castle sites were chosen based on previous data showing these sites to have high *Cx. pipiens* populations (O'Connor 2008). Glenville was shown to have above average *Culex* populations based on collections performed by the Mosquito Control Section of the Delaware Department of Natural Resources and Environmental Control (DNREC - unpublished data, 2008).

### **Rearing**

Water infused with grass clippings was conditioned for at least 72 hours to allow for decay of chlorides and other contaminants potentially harmful to the larvae (Gerberg 1970). This infusion water was placed in open containers (30x38cm) and set out at individual field sites overnight for approximately 12 hours. The vegetation used for the fermented water in 2008 was mixed clippings of grass but was switched to cut alfalfa in 2009 to improve attractiveness (Ritchie 1984). Mosquito egg rafts were collected on moist paper towels, labeled, and carefully transported back to the laboratory where rafts were placed in individual, labeled rearing trays of water. All

rafts were equally divided among three environmental chambers that contained pans with anthropogenic water sources for rearing at three different daylength settings, (L:D): 13:11; 14:10; and 15:9. All other environmental conditions were maintained at 27°C, 70% RH among the experimental groups until all larvae were reared. All larvae were maintained on a 3:1 liver powder: brewers'-yeast diet (Mori et al. 1988). All larval groups were identified to species and only *Cx. pipiens* were kept for continued use in this study.

Starting at approximately 6 days or at their first appearance, pupae were removed each day and placed in open emergence containers. These containers were placed in 30.5 cm<sup>3</sup> collapsible cages which housed adult mosquitoes that emerged. Moistened cotton towels were placed on the tops of cages to increase relative humidity. These towels were changed daily and washed regularly to avoid the build-up of mildew. Adult mosquitoes were sustained using cotton wicks soaked in 10% sucrose solution (Gerberg 1970). When possible, heparinized sheep blood (warmed to 21-23 °C in a hot water bath and trickled along the top of the cage using a pipette) was offered with the intention of inducing female mosquitoes to blood feed and oviposit as in a normal gonotrophic cycle (Knierim et al. 1955). In the event that females successfully fed on sheep blood, notation was made, and a container of infusion water was placed in the cage for females to oviposit. Females that were observed to have taken a bloodmeal were aspirated out of the population and kept in separate groups (of



approximately 40 individuals) for separate trials in a choice test feeding chamber (described below). Sucrose solutions were removed 24 hours prior to initiation of host choice testing.

### **Mosquito Host Choice Tests**

Emerging adults from laboratory-reared egg rafts were maintained as previously described. The sucrose solution supplement was removed 24 hours prior to host choice testing. Three days post-emergence and one hour before the end of the specific day-length among the three treatments being tested, mosquitoes were moved to the room in which host choice tests were performed. While mosquitoes were in the test-chamber, they were exposed to a specifically timed lighting regimen: one hour of full light, one hour of half light, and one hour of darkness. The host choice chamber was divided into three compartments (Figure 1). The end compartments contained physically restrained Japanese quail (*Coturnix japonica* Temminck and Schlegel) or golden Syrian hamster (*Mesocricetus auratus* Waterhouse) hosts while the middle compartment, which had a small operating fan for air flow, functioned as the release area for each mosquito cohort. Golden Syrian hamsters were placed within handmade cylinders of ½ inch metal wire mesh in order to confine hamsters in the center of the compartment and prevent escape. Host choice was documented not by feeding but by pre-feeding behavior: mosquitoes entering the compartment of a particular host were recorded as indicating host choice.

Host weight was monitored weekly to ensure comparability throughout use. Adult golden Syrian hamster weight averaged 110 g ( $\pm 3.8$ ) and remained constant for the season so that replacement animals were not needed. Quail weight ranged from 80 g to 125 g so that only quail weighing 110 g ( $\pm 5$ ) were used. This called for the replacement of quail populations at least once per season.

The number of individual mosquitoes used in each test-chamber ranged from 25-40 adult females. After the three hour light regimen, the final choice total in each chamber compartment was recorded; mosquitoes were aspirated back into holding containers and kept at the initial, specific light cycle conditions for two additional days after which the host choice test was repeated as before. Control tests for positional effect were performed regularly to ensure that mosquitoes were not inherently biased toward a particular end of the test chamber. This was done by carrying out a choice test as described above, but not inserting any hosts into either side of the test-chamber. All compartments of the chamber were cleaned using warm soapy water between trials. The host placed in each side of the choice chamber was also randomized, so that there was no chance that results were biased by a possible position effect. In separate host choice tests, mosquitoes were artificially fed on sheep blood and allowed to complete one gonotrophic cycle. The mosquito groups tested were all reared at 15 hours daylength and were over 21 days old.



**Figure 1**      **Choice Chamber.** Host Choice Chamber, showing the three compartments with example hosts placed in each side chamber. Here, hosts are restrained within stockings.

### **Seasonal Adult Collections Using Host-baited Traps**

Host-baited traps were set out at the field sites to assess the tendency for *Culex pipiens* to be attracted to either a mammal or a bird throughout the season. These traps involved the same animals used in the laboratory choice test, golden Syrian

hamsters and Japanese quail. Animal Use protocols (AUP # 1188-2008-0 and AACUC# (29) 04-14-08R) were followed using traps that were modified CDC light traps with an associated plastic cylinder housing the animal above a downdraft fan that created air flow, trapping mosquitoes in a collection container attached to the fan (Emord and Morris 1982) (Figure 2). Each night, one hamster and one quail trap were set out at a specified site concurrently and approximately 20 m apart. One animal was placed in each trap and each animal was provided with 254 cm<sup>2</sup> of floor space, 20cm of overhead space, and food and water containers. A 241 cm<sup>2</sup> screen with 1/4" mesh screen covered an opening in the floor of the container; sturdy 1/2" metal wire mesh was placed beneath hosts to prevent escape. Screening allowed air flow for the comfort of the animals and localized the attraction of mosquitoes close to the fan for capture. Traps were hung approximately 4 ft from ground and left out overnight from approximately 6 PM to approximately 8 AM the following morning, coinciding with peak *Cx. pipiens* feeding time. Collection containers of mosquitoes were brought into the laboratory and placed in a freezer for later identification.



**Figure 2** **Host-baited trap.** Pictured with animal housing container locked above fan and attached collection container (bottom).

### **Adult Collections Using Gravid Traps**

Gravid traps consisted of a converted tool box housing a battery-powered fan and an associated collection container (Bioquip, Rancho Domingo, CA). The tool box sat atop a flat, open plastic container used to hold grass-infused water. Gravid females were attracted to the nutrient-rich water and sucked into the collection container due

to the updraft air current created by the fan. These traps were placed out overnight at each collection site on a weekly basis. Collection containers were labeled, brought to the lab and kept in a freezer for later identification. All mosquitoes were identified to species, but only *Cx. pipiens* were used in the results recorded for this study.

### **ELISA: Determining Mosquito Bloodmeal Sources**

To convert the protein in blood to egg mass, gravid females require a bloodmeal. Through bloodmeal analysis, it was possible to determine the host source of these bloodmeals in individual gravid females. The gravid females obtained through the gravid trapping methods described above were used to determine seasonal trends in host preference. An IgG sandwich antibody ELISA was performed on all *Cx. pipiens* specimens collected using gravid traps to obtain such results. The assay involved the use of capture antibodies developed to bind specific IgG antigen domains found in blood samples of different animal hosts. This protocol was employed by Gingrich and Williams (2005) which was modified from Chow et al. (1993), including the reagents used and their sources (KPL Inc., Gaithersburg, MD). Costar 96-well plates (Corning Inc., Corning, NY) were used to screen for six possible hosts: deer, dog, cat, rabbit, human, and chicken. Chicken capture antibody was used as a general screening for bird blood-meals because this antibody is highly cross-reactive with bloodmeals of most bird species (KPL Inc.). An individual mosquito was macerated and tested against each of these capture antibodies as described by Gingrich and

Williams (2005). Mosquitoes that were laboratory-reared and known to have not been blood fed were macerated and served as the negative control. Positive controls were mixed using separate samples of the unfed laboratory mosquitoes spiked with purified sera from the designated animal hosts listed above. Following addition of ABTS substrate (KPL Inc.), plates were read using a SpectraMax 190 (Molecular Devices Corp., Sunnydale, CA) at 15 and 30 minutes post-incubation. A positive well was considered one with a value greater than the mean of the negative control wells by three times its standard deviation.

### **Avian Point Counts**

Point counts performed at each site of trap placement determined the relative abundance of bird species throughout the season. Point counts involved recording the bird species (seen or heard) and distance from observer in a 50m radius over a set time period of 10 minutes (Ralph et al. 1993). The counts were performed three times from 6 AM to 8:30 AM, biweekly throughout the data collection season. Longer count periods increase the chance of recording a single bird more than once, because the bird may move or the observer may forget its location (Scott and Ramsey 1981). Also spending longer times at a point increases the chance of recording new birds that move into the counting range from outside the survey area (Granholm 1983). These effects might influence the accuracy of estimated bird density (Shiu 2003).

## **Data Analysis**

Trials for host choice tests were separately analyzed based on the age of the adult mosquito cohort (in days) or the daylength during larval rearing. Repeated G-tests (McDonald 2009) were run to determine if there were significant differences within treatment groups. Standard errors were calculated for each grouping of tests. The results of host-baited traps were analyzed seasonally. These were separated into three seasonal cohorts to represent different daylength patterns: June 6 to July 16 (14.58-14.42 hours); July 17 to August 4 (14.42 to 13.58 hours); and August 5 to September 10 (13.58-13.05 hours). Natural log transformations of trap data were calculated to obtain a normal distribution from data skewed to the right. Paired two-tailed t-tests were used to determine significant differences between the number of mosquitoes attracted to the quail and hamster traps for each seasonal division. Logistic regression (SAS Institute Inc. 2008) was performed with mosquitoes entering hamster traps set as the event. Mosquitoes entering the hamster trap were analyzed because unlike the trend of mosquitoes entering the quail trap, there appeared to be significant trend with respect to the interaction variables available. Therefore, interactions for site, seasonal week, and year were modeled. ELISA data were analyzed using logistic regression (SAS Institute Inc. 2008) to determine changes in the probability of a mammalian bloodmeal based on collection year, seasonal week, and site.

Avian point count abundances were corrected using Distance software (Thomas et al. 2010). Model definitions were set as described in Somershoe et al.



(2006). New estimated abundance (N) was calculated as:

$$N=x/p$$

Here, x is the original observed abundance and p is the detection probability

(Farnsworth et al. 2002).

## RESULTS

### Mosquito Host Choice Test

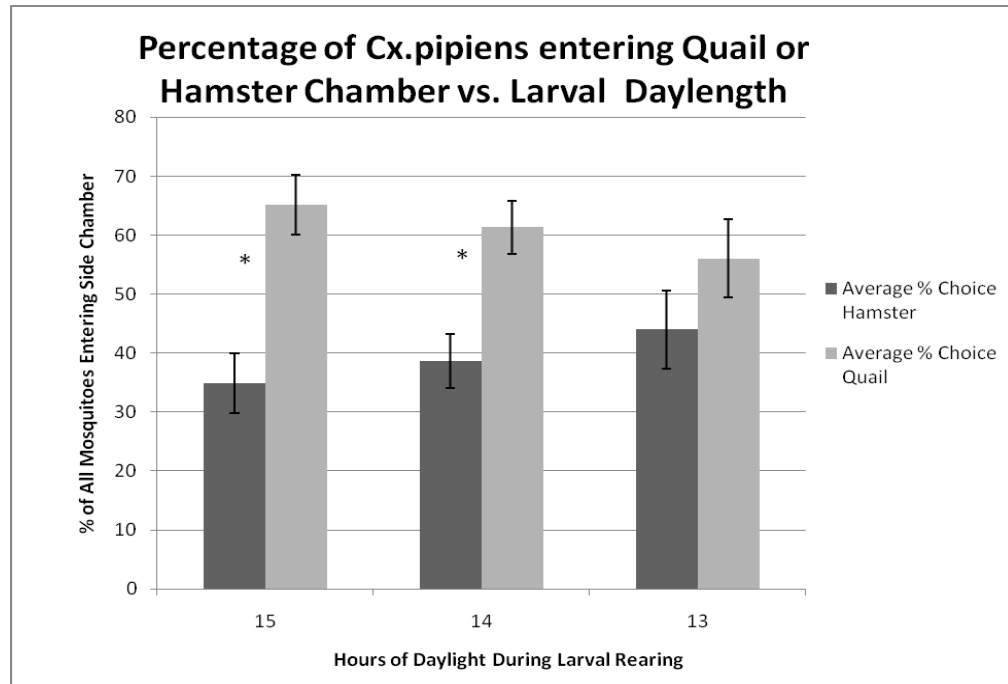
A total of 63 host choice tests were performed using Japanese quail and golden Syrian hamster hosts exposed to adult mosquito cohorts reared at three different treatment photoperiods. Table 1 shows that of the three treatment types, the mosquito cohorts reared at 13 hours of daylight were significantly more likely to enter any of the host side chambers at a mean of 49.1 % (standard error  $\pm$  6.5).

**Table 1**     **Choice Test: Daylength Cohort Results.** Mean percent of mosquitoes entering any side chamber for each of the larval rearing daylength exposure patterns: 15 hours; 14 hours; 13 hours

<b>Group (Hours of Daylight/Day)</b>	<b>n</b>	<b>Mean % Choice</b>	<b>St. Error</b>
15	24	39.9	5.2
14	16	36.4	4.5
13	23	49.1	6.5

An average between 36% and 49% of mosquitoes in each cohort entered any side chamber during choice testing (Table 1). A significantly higher percentage of mosquitoes entering any side chamber entered the quail chamber for all cohorts reared under 15 and 14 daylight hour treatments. This statistical significance was not

observed when testing mosquito cohorts reared at the 13 hour treatment (Figure 3).



**Figure 3 Choice Test by Daylength Treatments.** Mean percent of mosquitoes entering the quail or hamster side chamber compared for cohorts that experienced the same rearing daylength exposure patterns: 15 (n=24), 14 (n=16), and 13 (n=23) daylight hours (\* denotes statistical significance using G-test of independence where  $p=7.7E-06$  for 15 hours and  $p=2.0E-08$  for 14 hours, McDonald 2009).

Groups of adult mosquitoes were tested in the choice chamber at different age groups. When pooled, all age groups had approximately equal numbers of individual

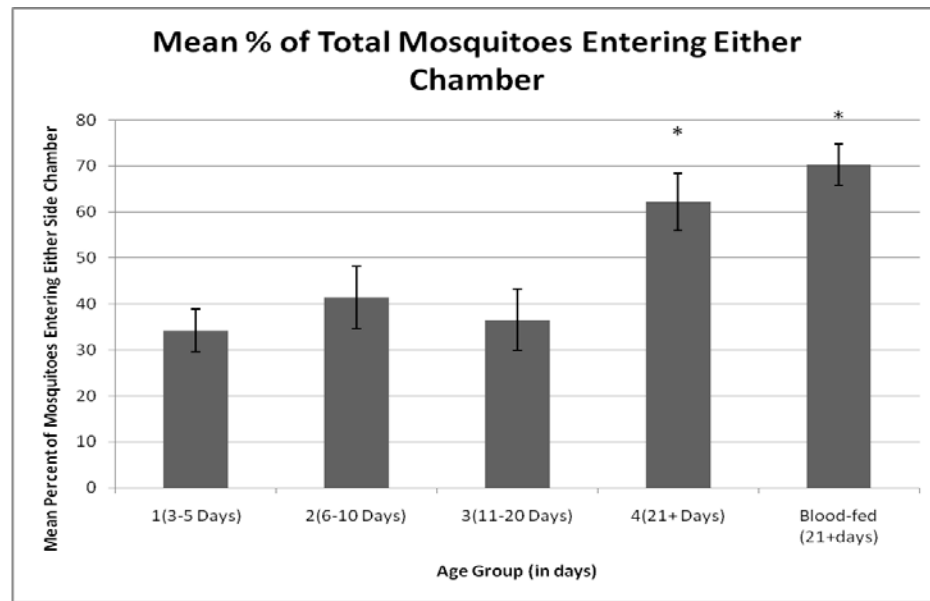
cohorts reared at all photoperiod treatments. The oldest group, reaching 21+ days, had the lowest n values due to difficulty of keeping large groups alive for extended periods of time. The youngest mosquitoes in age, 3-5 days, showed the lowest mean to enter any host side chamber at 34.2 % ( $\pm$  4.7) (Table 2). The mosquitoes aged to 21+ days exhibited the highest mean % to enter any side chamber at 62.2 % ( $\pm$  6.2).

**Table 2 Choice Test: Age Cohort Results.** Mean percent of mosquitoes entering any side chamber for each of the adult mosquito age groups.

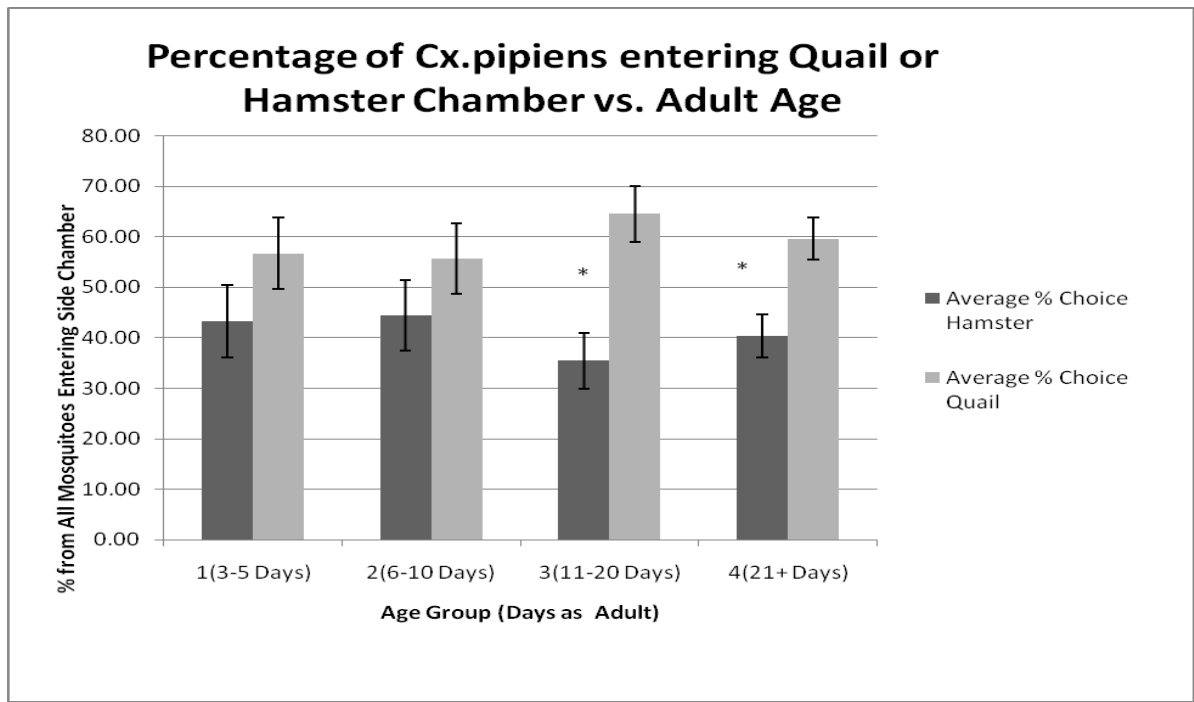
<b>Group (By Age)</b>	<b>n</b>	<b>Mean % Choice</b>	<b>St. Error</b>
1(3-5 Days)	14	34.2	4.7
2(6-10 Days)	18	41.4	6.8
3(11-20 Days)	14	36.6	6.7
4(21+ Days)	10	62.2	6.2
Blood-fed (21+days)	15	70.3	4.5

Mosquitoes were artificially fed on sheep blood and allowed to complete one gonotrophic cycle. These blood-fed mosquitoes exposed to the host chamber tests showed a mean of 70.3 % ( $\pm$  4.5) mosquitoes entering either side chamber (Table 2). The mosquito groups tested were all reared at 15 daylight hours and were in the 21+day age group. Of the mosquitoes that did enter a side chamber, 52.4 % ( $\pm$ 3.3) entered the quail side chamber and 47.6 % ( $\pm$ 3.3) entered the hamster side chamber

(repeated G-test  $p=0.52$ ). Overall, the mosquito cohorts that reached 21+ days had statistically higher mean percent mosquitoes entering any side chamber. This was also true for groups that were artificially bloodfed (Figure 4).



**Figure 4** **Choice Test Age Groups.** Mean percent of mosquitoes entering either side chamber for groups the same age in days. The last x-axis value represents mosquitoes that were tested after artificial blood-feeding (\* denotes age cohorts with a mean percent statistically different from those lacking an \*, using G-test of independence where  $p=1.2E-03$  for age group 4 and  $p=2.2E-05$  for bloodfed mosquitoes, McDonald 2009).



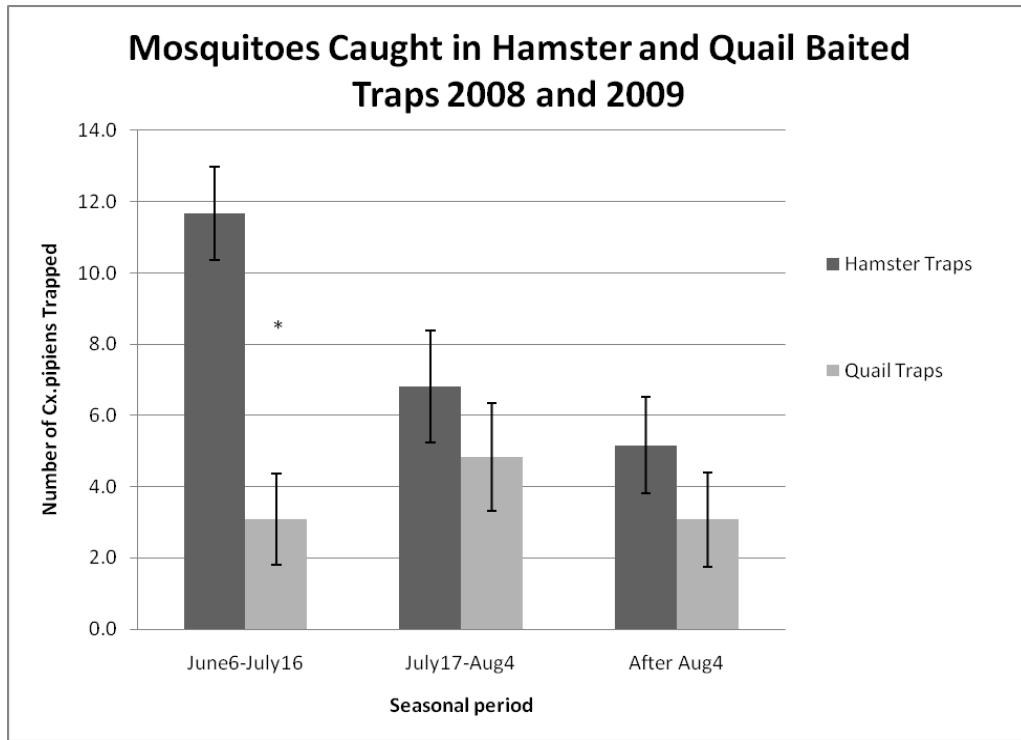
**Figure 5 Choice Test by Age Group Separating Mean Host Choice.** Mean percent of mosquitoes entering the quail or hamster side chamber compared for age (\* denotes statistical significance using G-test of independence where  $p=1.3E-09$  for group 3 and  $p=2.7E-06$  for group 4, McDonald 2009).

Of the mosquitoes to enter a side chamber, there was no statistical difference between the mean percent entering either the quail or the hamster side chamber for age groups reared at 3-5 days or 6-10 days. Groups reared at 11-20 days had a higher mean percent of mosquitoes entering the quail chamber over the hamster chamber at 64.5 % ( $\pm 5.5$ ) and 35.5 % ( $\pm 5.5$ ) respectively. This was also seen in the mosquito

groups 21+ days old where a mean of 59.6 % ( $\pm 4.2$ ) mosquitoes entered the quail side chamber and a mean of 40.4% ( $\pm 4.2$ ) entered the hamster side chamber (Figure 5).

### **Adult Collections Using Host-baited Traps**

The field CDC trapping results were separated into three seasonal periods to mirror natural progression in daylength patterns. These periods were as follows: (1) June 6-July 16; (2) July 17-August 4; and (3) after August 4th. The mean number of mosquitoes entering the hamster trap ranged from 11.7 ( $\pm 1.3$ ) from June 6 to July 16 to 5.2 ( $\pm 1.4$ ) after August 4. The mean number of mosquitoes entering the quail trap peaked at 4.8 ( $\pm 1.5$ ) from July 17- August 4th (Figure 5). Natural log transformation was performed on values before statistical testing. The only statistical difference in number of mosquitoes trapped by the hamster and quail traps was found from June 6-July 16 (Figure 6).

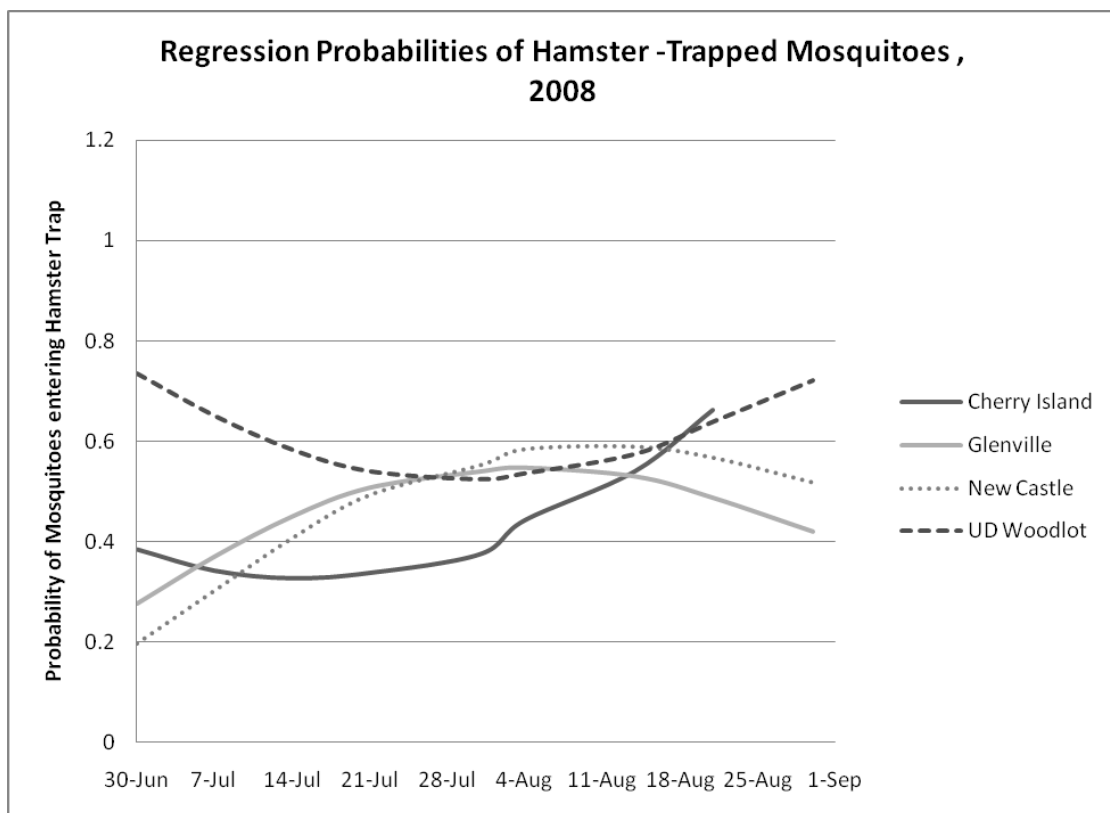


**Figure 6 Host-baited Trapping Results.** The number of mosquitoes trapped in quail-baited and hamster-baited CDC traps over seasonal periods: June 6-July 16 (n=21), July 17-August 4 (n=11), and After August 4 (n=18) (\* denotes statistically significant difference using two tailed paired t-test  $p=3.0E-04$ ).

No statistical significance was seen in the number of mosquitoes entering quail traps with respect to interference variables. However, there were significant trends occurring with the number of mosquitoes trapped in hamster traps. Logistic regression was performed setting mosquitoes entering hamster traps as the event as

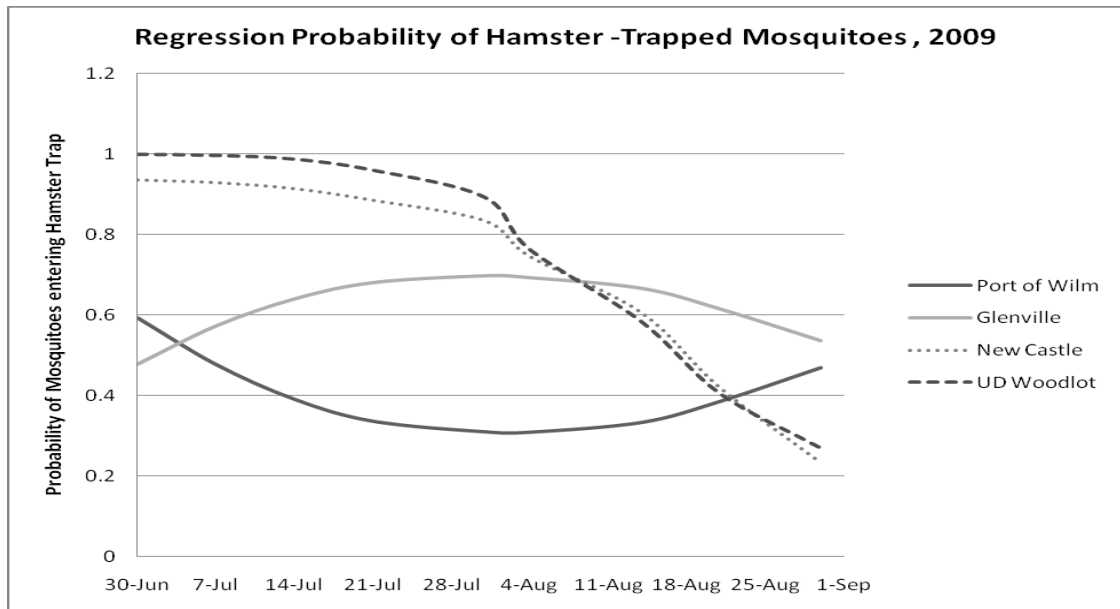


this behavior is of particular interest. Interactions were analyzed with predictor variables: site, seasonal week, and year. Figure 7 shows the regression trends for 2008. Cherry Island had a general increase in probability of a mosquito entering a hamster baited trap throughout the season while both Glenville and New Castle sites exhibited a peak probability from July 31- August 10. The inverse of these relationships was seen with the probabilities at the UD Woodlot, which actually reached its lowest point on July 31. UD Woodlot displayed the highest probabilities, approaching 0.80, in late June and late August.



**Figure 7 Regression Probabilities for Host-Baited Trapping, 2008.** Probability of mosquitoes entering a hamster-baited trap vs. quail-baited trap for each site throughout the 2008 season.

The regression probabilities calculated for hamster-baited traps in 2009 are shown in Figure 8. New Castle and the UD Woodlot showed a general decline through the season and registered the higher probabilities of mosquitoes entering mammal-baited traps at or near 1.00 around July 1. Glenville peaked in probability of mosquitoes entering mammal-baited traps around July 31, while probabilities at the Port of Wilmington reached the lowest values at this same point.



**Figure 8 Regression Probabilities for Host-Baited Trapping, 2009.** Probability of mosquitoes entering hamster-baited trap versus quail-baited trap for each site throughout the 2009 season.

**ELISA: Determination of Mosquito Bloodmeal Sources**

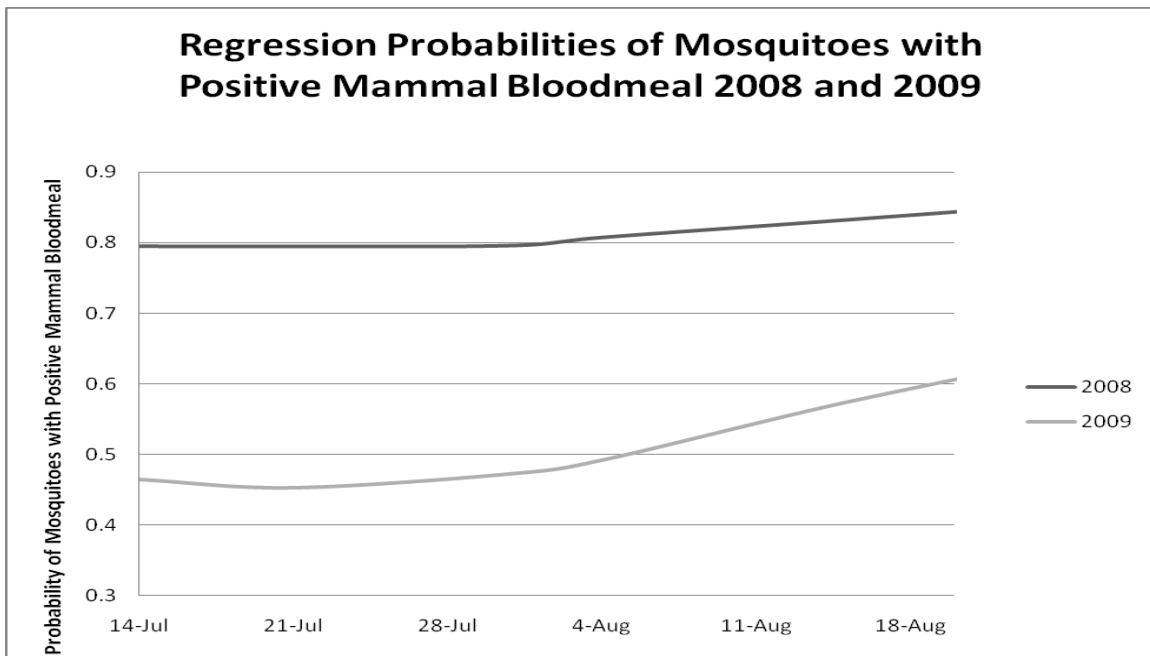
As seen in Table 3, the percentages of mosquitoes, collected from June 6 to July 16, testing positive for avian and mammalian bloodmeals were similar at 44.8% and 55.2% respectively. This picture largely changed when looking at the percent of mosquitoes, collected after August 4, testing positive for avian and mammalian bloodmeals, which were 30.1% and 69.1% respectively. The number tested grew over the seasonal periods from 287 (June 6 to July 16) to 388 (after August 4), while the number that tested positive for any of the host antibodies used fell from 96 to 68 during the same seasonal periods.

**Table 3 ELISA Bloodmeal Analysis Results.** Total number and percent of mosquitoes positive for bird and mammal bloodmeals for each seasonal period pooled data for 2008 and 2009 seasons.

	June 6- July 16	% from Total Positive	July 17-Aug 04	% from Total Positive	After Aug 04	% from Total Positive
# Mammal Positive	53	55.2	29	63.0	47	69.1
# Bird Positive	43	44.8	17	37.0	21	30.9
Total # positives	96		46		68	
Total # Tested	287		311		388	

<b>% Positive of Total Tested</b>	33.4	14.8	17.5
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The number of human blood meals ranged from 8 early in the season to 6 in late summer and early fall (Table A2). Regression tests revealed no difference in the probability of a mammalian bloodmeal as a function of site or week. However, mammalian bloodmeals were more probable in the 2008 season than in 2009 (regression;  $p=0.004$ ) (Figure 9).

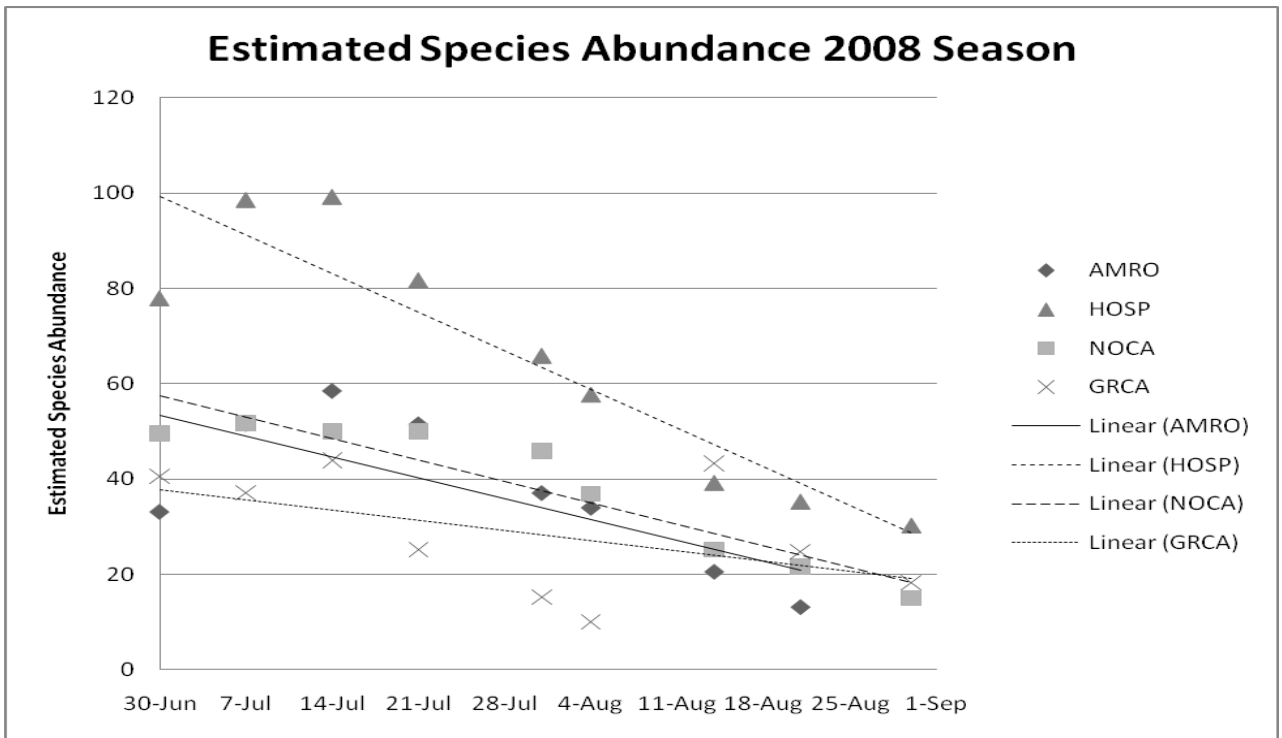


**Figure 9 Regression Probabilities for ELISA Results.** Seasonal probabilities of mosquitoes taking a mammalian bloodmeal for 2008 and 2009 (years are significantly different,  $p=0.004$ ).

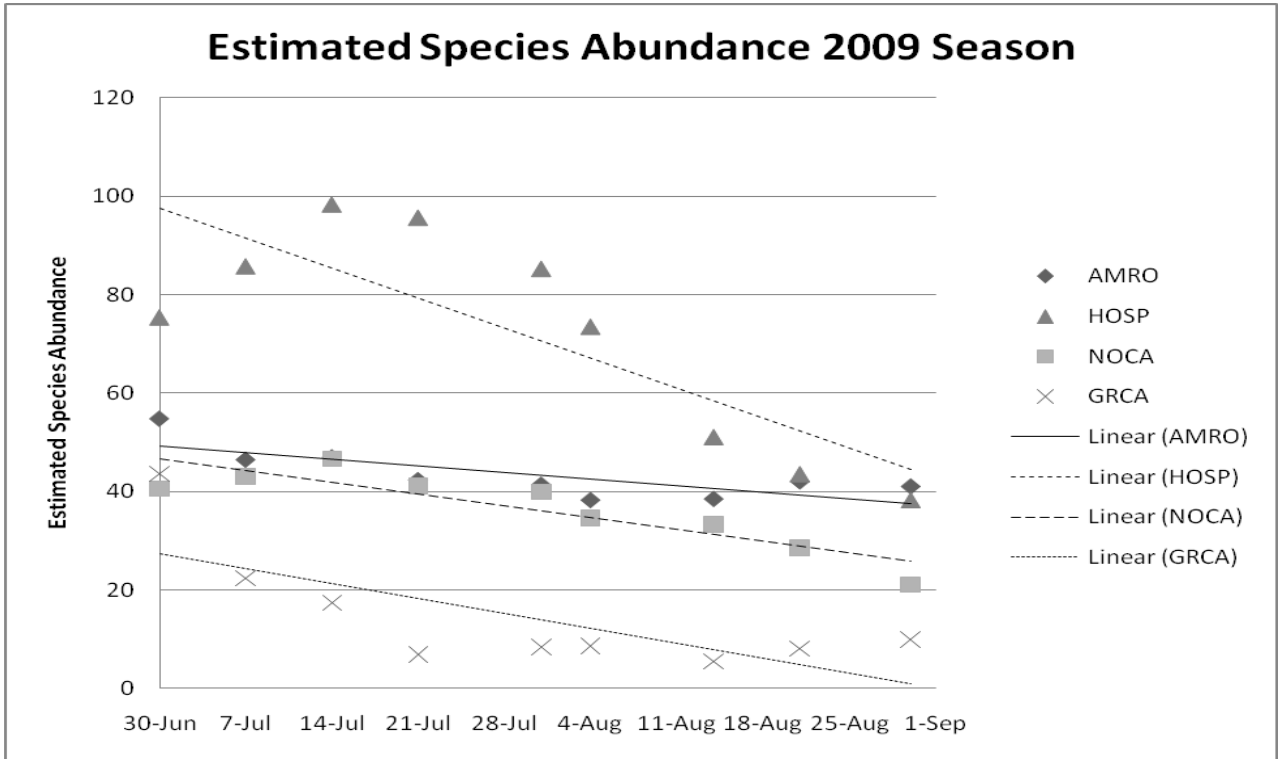
### **Avian Point Counts**

The four most common bird species recorded throughout the 2008-2009 seasons were the house sparrow (*Passer domesticus* L.), American robin, grey catbird (*Dumetella carolinensis* L.) and northern cardinal (Table A1). In 2008, estimated abundances of house sparrows, American robins, and northern cardinals largely declined from late July throughout August with house sparrows declining from 99.2 to 30.3. Northern cardinal abundance dropped from 51.7 to 14.9 during the same period. Estimated abundance of American robins fell 58.5 to 13.1 from late July to late August. Grey catbirds did not show a clear correlation between abundance and seasonal progression (Figure 10).

In 2009, the house sparrow population peaked at 98.3 in mid-July and steadily decreased through the end of August, 2009. Concurrently, the northern cardinal population fluctuated from 46.6 to 18.9, the American robin population ranged from 38.9 to 24.1, and the grey catbird population declined from 43.6 to 9.9 (Figure 11).



**Figure 10** Estimated Bird Abundance Values for 2008. AMRO – American robin, HOSP – house sparrow, NOCA – Northern cardinal, GRCA – grey catbird. Values are corrected, estimated bird abundances for each date.



**Figure 11** Estimated bird abundance values throughout 2009. AMRO – American robin, HOSP – house sparrow, NOCA – Northern cardinal, GRCA – grey catbird. Values are corrected, estimated bird abundances for each date.

## DISCUSSION

The various testing methods used in this study reveal several key components of *Culex pipiens* feeding that have not been previously observed. The chamber host-choice tests resulted in a statistically higher mean percent of mosquitoes entering the quail chamber at 15 and 14 daylight hours as compared to the hamster chamber. This statistical significance was lost when using mosquitoes reared at 13 daylight hours (Figure 3). As discussed, several authors have reported a gradual decrease in host specificity from the early to mid-summer months (Andreadis et al. 2004, Kilpatrick et al. 2006, Marshall et al. 2006, Patrican et al. 2007). The choice-test results reported here validate this trend as host specificity was significantly ornithophilic among cohorts exposed to 15 hour daylength treatments (peak summer) but not significantly different among cohorts exposed to other daylength treatments (mid- to late summer). Correlation of feeding behavior with daylength patterns during larval rearing has not been previously reported; however, these results implicate daylength as one possible cue affecting adult seasonal feeding behavior. This may be significant as previous studies cited have largely speculated that bird host abundance is the main cue for changes in *Culex* seasonal feeding patterns (Kilpatrick et al. 2006; Marshall et al. 2006; Savage et al. 2007).

This study also confirms O'Connor et al.'s (2009) report that mosquitoes fed more readily on hosts, regardless of species, as mosquito age increased (Figure 4).



Previously, methods allowed mosquito cohorts to age without any bloodmeal, whereas this study included a mosquito cohort that was artificially fed and completed a gonotrophic cycle prior to testing in the choice chamber. Naturally, it is unlikely that a non-gravid mosquito would reach 20 days in age without having a previous bloodmeal. Therefore, the fifth cohort of mosquitoes was artificially fed sheep blood and completed one full gonotrophic cycle prior to testing in the choice chamber. As seen in Figure 4, these aged mosquitoes exhibited similar feeding behavior to the 20-day old mosquitoes that did not have a previous bloodmeal. O'Connor et al. (2009) also reported on the age structure of *Culex* populations throughout the season, noting that the overall population increases in age as summer progresses. Higher rates of host choice with increasing age and decreasing larval photoperiod have implications for arboviral disease transmission. It seems that the peak mean percentages of host choice found from these study methods mimic the naturally-occurring conditions late in the season. Several locally endemic arboviruses, including West Nile virus, circulate within the avian population throughout early- to mid-summer with epizootic infections occurring in the late summer and early fall (Nasci and Moore 1998).

Although molecular identification was not performed on all specimens to determine conclusive identity of mosquitoes within the *Cx. pipiens* complex, it is highly unlikely that hybridization with *Cx. pipiens* f. *molestus* affected the results of laboratory host chamber tests. Eff rafts were collected from large, openly accessible

breeding containers that would not serve as likely sites for *Cx. pipiens f. molestus* breeding. Also, in previous work by O'Connor (2008), molecular signatures of mosquitoes collected in the same region as the sites used in this study revealed no correlation with host feeding behavior.

Host-baited trapping techniques performed in the field were intended to validate the laboratory controlled host choice tests. However, the results were surprising in that the natural log transformations of the number of mosquitoes trapped revealed a statistically higher level of hamster trappings early in the season (Figure 6). As with any field study, there are several components that are difficult to quantify, including mosquito distance from hosts while seeking and the abundance of naturally-occurring, competing hosts.

The more striking evidence provided by the host-baited trap study reveals the pronounced differences in trapping probability among field sites. Initially we hypothesized that previous studies reported conflicting trends in feeding behavior because of differences in geographic location. However, the regression probability trends shown in Figures 7 and 8 reveal striking differences in seasonal hamster-trapping capacity for all field sites and for individual field sites between years. This finding diminishes the influential strength of biological cues such as age and rearing photoperiod as strong cues for *Cx. pipiens* host choice behavior because these factors

were similar for all the sites used. The drastic differences observed may instead implicate more irregular cues such as host abundance as a more influential factor.

The ELISA bloodmeal analyses more directly tested the successful feeding behavior of mosquitoes in the field. ELISA results showed an increase in percentage of mammalian feeds throughout the season, which corresponds with the previous literature using similar methods of analysis (Kilpatrick et al. 2006, Hamer et al. 2009). This study reports a percentage of mammalian bloodmeals at 69% of meals after August 4 (Table 3). This percentage is comparable to that reported by Hamer et al. 2009 with 56% combined human and non-human mammalian bloodmeals. However, Kilpatrick et al. (2006) reported a September peak in mammalian feeding of over 40% of bloodmeals which is substantially lower than the 69% reported here. Logistic regression revealed the only statistically significant interaction with the incidence of mammalian bloodmeals was year, with a higher probability of bloodmeals occurring in 2008 (Figure 9).

There were compelling differences between the results of the three testing methods: host choice, host-baited trapping, and ELISA bloodmeal analysis. At the onset of this study, we hypothesized that all results would reflect the central pattern of *Culex pipiens* host-switching from avian to mammal hosts from mid-summer to early fall. This pattern was reflected in the results of the laboratory host choice tests where mosquitoes raised under conditions mimicking peak summer and fall photoperiods

separately showed a decrease in host specificity (Figure 3). However, the host-baited trapping methods revealed a higher tendency for mosquitoes to enter mammal traps early in the season, while the number of mosquitoes entering quail-baited and hamster-baited traps late in the season lacked a statistically significant difference (Figure 6). Also, the pooled percentage of mammal and avian mosquito bloodmeals were about equal from June 6 to July 16, yet the percentage of mammalian bloodmeals doubled that of avian bloodmeals in the July 16- August 4 time period. This indicated an increase in the percentage of mammalian bloodmeals taken throughout the season (Table 3). These discrepancies most likely reflect the differences in methods used. For instance, the host choice is performed in controlled conditions with hosts in close proximity to the mosquito population, while both the host-baited traps and ELISA methods rely on dispersed mosquito populations in the field and are influenced by natural host populations that are often difficult to accurately assess. While host-baited trapping and ELISA methods were both associated with the field, host-baited trapping reveals only the seeking of hosts, while ELISA results reveal true success in feeding. These differences may account for the differences in results from the three testing methods.

Both Kilpatrick et al. (2006) and Hamer et al. (2009) tied feeding preference to avian host abundance. These correlations are dependent on seasonal trends in avian abundance. Kilpatrick et al. (2006) reported a general decrease in American robin

abundance, which the authors indicate as the preferred host of *Cx. pipiens*. Hamer et al. (2009) similarly associated a drastic drop in American robin abundance directly to a peak in human bloodmeals. Unlike these previous studies, bird species abundance was separately analyzed for the 2008 and 2009 seasons in this study. Separation of data by year shows the confounding issue of changes in bird abundance trends between years. Estimated abundances were used to plot the trend in species abundance throughout the season. Figure 10 (representing 2008) shows a similar trend as that reported in both Kilpatrick et al. (2006) and Hamer et al. (2009). The abundance of key host species such as American robins and northern cardinals began to decrease in early August. This is a common trend shown reported in previous studies (Kilpatrick et al. 2006). The decrease in estimated abundance of bird species was not as drastic throughout the 2009 season (Figure 11). Therefore, the importance of correlating bird abundance and mosquito feeding behavior for a single season and for individual sites must be stressed.

Bird species may migrate out of a particular region if it is an unsuitable overwintering environment (Doherty and Grubb 2000). This may explain the weak trend observed in the 2009 species abundance data (Figure 11). While common species such as house sparrows showed a decline through the season similar to 2008, neither American robins nor northern cardinals presented a comparable decline in abundance through the late summer (Figure 11).

When compared to ELISA data, the bird abundance trends seem to link *Cx. pipiens* host choice to host abundance. In 2008, when American robin and Northern cardinal species experienced a large decline in numbers later in the season (Figure 10), the seasonal probability of mammalian ELISA positives was statistically higher (Figure 9). In 2009 where a weak declining trend in the abundance of host bird species was recorded (Figure 11), seasonal probability of mammalian ELISA positives was lower (Figure 9). This suggests an increase in successful mammalian bloodmeals may be coupled with a decline in avian host species abundance. Several of the data collection sites went through a change in landscape structure between the 2008 and 2009 seasons. A road directly adjacent to the Glenville site had high traffic of construction vehicles for much of the 2008 season, but was abandoned and unmaintained throughout the 2009 season. The road itself became overgrown with undergrowth vegetation. Similar changes occurred at the New Castle site to a lesser extent. These changes may have affected the tendency for populations of American robins and northern cardinals to overwinter locally, which may explain the differences in seasonal bird observations between the 2008 and 2009 seasons. Landscape structure has been correlated to bird abundance, density, and species richness where fluctuations in habitat size and understory vegetation are linked to change in overwintering bird populations (Doherty and Grubb 2000). Landscape fluctuations

such as those described could also provide resting areas for mosquito populations, but this was not reflected in trapping data.

Overall, the controlled laboratory choice tests revealed significant correlations between *Cx. pipiens* feeding behavior and biological cues such as mosquito age and larval photoperiod exposure that have not been previously reported. Further investigation is needed to determine the impact of these predictors on *Cx. pipiens* feeding in the field. It is likely that these cues act upon feeding behavior which is more generally modulated by host availability. While the host-baited trapping was largely successful in attracting *Cx. pipiens* throughout the season, these data must be coupled with a better understanding of the naturally occurring avian and mammalian host populations during the trapping period. This should enable a better understanding of how trapping results relate to *Cx. pipiens* host-seeking behavior in the field. Lastly, the ELISA results on mosquito bloodmeals and bird species abundance data reported here generally correspond with previously reported trends in *Cx. pipiens* feeding behavior as it pertains to preferred avian host availability. This study would have been more informative if ELISA was performed specific to common avian hosts such as American robins and northern cardinals because this relationship may correlate with bird abundance data to support the hypothesis that *Cx. pipiens* feeding is strongly dependent on preferred avian host availability.

## APPENDIX

**Table A1 Observed Bird Count Totals for 2008 and 2009.** Totals include only sites that were consistently monitored for both seasons: Glenville, New Castle, and UD Woodlot.

2008-2009	Seasonal Total	Percent of Total
House Sparrow ( <i>Passer domesticus</i> )	189	29.62
American Robin ( <i>Turdus migratorous</i> )	92	14.42
Grey Catbird ( <i>Dumetella carolinensis</i> )	82	12.85
Northern Cardinal ( <i>Cardinalis cardinalis</i> )	80	12.54
Common Grackle ( <i>Quiscalus quiscula</i> )	27	4.23
Mourning Dove ( <i>Zenaida macroura</i> )	24	3.76
European Starling ( <i>Sturnus vulgaris</i> )	19	2.98
Pileated Woodpecker ( <i>Dyrocopus pileatus</i> )	17	2.66
American Goldfinch ( <i>Carduelis tristis</i> )	17	2.66
Tufted Titmouse ( <i>Parus bicolor</i> )	15	2.35
Purple Martin ( <i>Progne subis</i> )	15	2.35
Indigo Bunting ( <i>Passerina cyanea</i> )	11	1.72
House Finch ( <i>Carpodacus mexicanus</i> )	8	1.25
Brown-headed Cowbird ( <i>Molothrus ater</i> )	7	1.10
Carolina Wren ( <i>Thryothorus ludovicianus</i> )	7	1.10
Song Sparrow ( <i>Melospiza melodia</i> )	5	0.78
American Crow ( <i>Corvus brachyrhynchos</i> )	5	0.78
Blue Jay ( <i>Cyanocitta cristata</i> )	4	0.63
Northern Flicker ( <i>Colaptes auratus</i> )	4	0.63
Eastern Phoebe ( <i>Sayornis phoebe</i> )	3	0.47
Summer Tanager ( <i>Piranga rubra</i> )	3	0.47
House Wren ( <i>Troglodytes aedon</i> )	3	0.47
White-breasted Nuthatch ( <i>Sitta carolinensis</i> )	1	0.16
	638	



**Table A2 ELISA Bloodmeal Positives for all Antibodies Tested.**

<b>Antibody Type</b>	<b>June 6- July 16</b>	<b>% from Total Positive</b>	<b>July 17- Aug 04</b>	<b>% from Total Positive</b>	<b>After Aug 04</b>	<b>% from Total Positive</b>
<b>Deer</b>	11	11.5	6	13.0	10	14.7
<b>Dog</b>	8	8.3	0	0.0	4	5.9
<b>Cat</b>	12	12.5	5	10.9	8	11.8
<b>Rabbit</b>	12	12.5	9	19.6	12	17.6
<b>Human</b>	8	8.3	3	6.5	6	8.8
<b>mixed mammal</b>	2	2.1	6	13.0	7	10.3
<b>Bird</b>	36	37.5	17	37.0	20	29.4
<b>mixed w/bird</b>	7	7.3	0	0.0	1	1.5

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